2003P Expression of mutant p53 affects cancer cell sensitivity to topotecan

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Background: Inactivation of tumor suppressor p53 is a common event in tumor progression. In almost 50% of cases p53 inactivation is caused by mutations that primarily affect DNA-binding domain. Oncogenic missense mutation Y220C is the ninth most common for p53 and is annually observed in ~100,000 new diagnosed cancer cases worldwide. Presence of this mutation disturbs tertiary structure of the p53 DNA-binding domain that further leads to destabilization of the whole protein, its partial denaturation and loss of transcriptional activity. In some cases, p53 mutations result in more aggressive cancer cells and alter drug sensitivity.

Methods: We employ CRISPR-Cas9 gene editing technology to generate p53 knockout and Y220C mutant MCF-7 cell lines. Quantitative analysis of alterations in intracellular protein levels were performed by immunoblotting, analysis of p53-dependent gene expression by quantitative real-time reverse transcription PCR; cell proliferation and chemotherapy cytotoxicity by MTS test. Statistical analysis was performed using ANOVA and Holm-Sidak method for multiple comparison, $p \leq 0.05$ was considered to be statistically significant.

Results: We have confirmed that p53 mutation Y220C leads to p53 inactivation. Proliferation rate of p53 knockout and mutant MCF-7 cell lines was 15% higher than wild type. We have shown statistically significant decrease in topotecan cytotoxicity towards knockout and mutant cells compared to wild-type – 14% and 26%, respectively.

Conclusions: Decline of topotecan cytotoxicity in mutant and knockout cells can be explained by topotecan-mediated induction of p53 that leads to higher levels of cell death in wild-type MCF-7. References Bulatov, E., Zagidullin, A., Valiullina, A., Sayarova, R., Rizvanov, A. (2018). Small molecule modulators of RING-type E3 ligases: MDM and Cullin families as targets. Frontiers in pharmacology, 9, 450.

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